

# Effects of Parent Age at Mating on Reproductive Response of *Glyptapanteles flavicoxis* (Hymenoptera: Braconidae), a Larval Parasitoid of the Gypsy Moth (Lepidoptera: Lymantriidae)

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**ABSTRACT** *Glyptapanteles flavicoxis* (Marsh) (Hymenoptera: Braconidae) is a gregarious larval parasitoid of the Indian gypsy moth *Lymantria obfuscata* (Walker) (Lepidoptera: Lymantriidae), that is believed to have potential for inundative releases against gypsy moth populations, because it can be reared in large numbers with few hosts. Unfortunately, sex ratios in laboratory reared *G. flavicoxis* are usually male-biased, hindering efforts to mass release this species for biological control by making the production of females costly. Because parental age at time of mating is known to affect the sex ratio in some Braconidae, we crossed haploid males and virgin females at 0, 1, 4, 9, and 16 d old with at least 10 trials for each of the 25 combinations. Numbers and sex ratios of progeny produced by females each day were recorded. Both progeny and sex ratios (percentage of females) among progeny produced by ovipositing females of *G. flavicoxis* decreased markedly over time, so only the first days production need be used in mass rearing. The reduction in the proportion and numbers of females among progeny as females aged is consistent with sperm depletion. Approximately 30% of females in all age classes mated to newly emerged males (day 0) produced all male progeny, whereas only 10–15% of those mated to older males failed to produce any daughters. When crosses with only male progeny were excluded from the analysis, females mated to males 1 d old had higher sex ratios in progeny than those mated to males in other age classes. In addition, females mated the day that they emerged tended to have progeny with the highest sex ratios.

**KEY WORDS** parasitic Hymenoptera, sex ratio, sex determination, biological control

*Glyptapanteles flavicoxis* (Marsh) (Hymenoptera: Braconidae) is a gregarious larval parasite of the Indian gypsy moth *Lymantria obfuscata* (Walker) (Lepidoptera: Lymantriidae). Earlier laboratory studies showed that *G. flavicoxis* readily attacked the gypsy moth (Fuester et al. 1987), and releases of this species were made in the Middle Atlantic States, but they did not result in its establishment (Krause et al. 1991). Because of its gregarious development, large numbers of this parasitoid can be reared with relatively few hosts, and the species was considered to have potential for inundative releases directed at specific gypsy moth populations (Krause et al. 1991). In fact, *G. flavicoxis* was reared and released as part of a successful urban forest integrated pest management (IPM) program for gypsy moth in Virginia, but *Bacillus thuringiensis* (Bt) and releases of *C. melanoscelus* also were used in the program, so it is difficult to say how much *G. flavicoxis* contributed to the suppression effort (Ticehurst and Finley 1988). Unfortunately, sex ratios in laboratory rearings of *G. flavicoxis* are usually male-biased (Hu et

al. 1986a, 1986b; Fuester et al. 1987). Male-biased sex ratios hinder applied biological control efforts using parasitic Hymenoptera, because 1) a reduced number of female founders decreases the likelihood of establishment of imported species used in classical biological control, or 2) they make the production of females too costly for economical use in an augmentative approach. Many factors are known to contribute to male-biased sex ratios in the Braconidae, including among others: parental age at time of mating (Rungrojwanich and Walter 2000), host size or stage (Pandey and Singh 1998, Kraaijeveld et al. 1999), host species (Duan et al. 1998), maternal crowding (Biswas and Singh 1995), sex ratio of parents (Zaki et al. 1994), superparasitism (Gul and Gulel 1995), temperature (Whiting and Anderson 1932), and production of diploid males (Stouthamer et al. 1992). Some information is available for *G. flavicoxis*. Sex determination in this wasp is arrhenotokous, a system in which fertilized (diploid) eggs give rise to female progeny, and unfertilized (haploid) eggs, male progeny. In laboratory studies by using *L. dispar* as the host, Hu et al. (1986a) found that the sex ratio (expressed as % female progeny) declined with parental female age, but that the adult parental sex ratio (male:female ratio placed in mating

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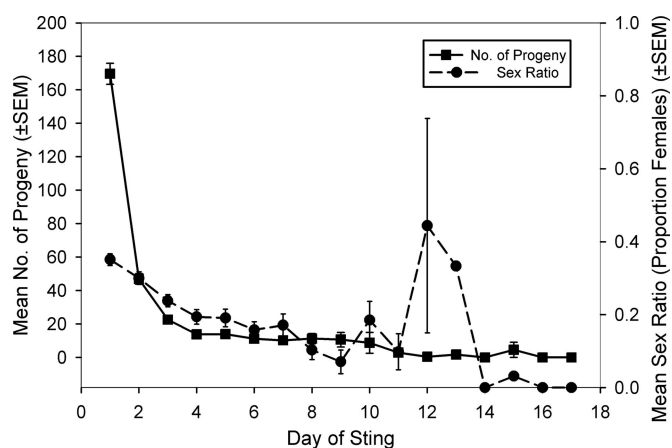


Fig. 1. Mean daily production of progeny and sex ratios of progeny produced by females over their lifetimes. Error bars denote standard error of the estimate.

cage, not observed sex ratio among emergees in parental generation), density of females in mating cages, and duration of time spent in mating cages (24 or 48 h) did not affect sex ratios (Hu et al. 1986b). Fuester et al. (1987) made laboratory studies on the reproductive response of *G. flavicoxis* to different instars and densities of *L. dispar* and found that host density had no effect on sex ratios, but that sex ratios in progeny of females provided fourth and fifth instar hosts were higher than those offered second and third instars. Although these studies provided useful information on factors influencing sex ratios, progeny obtained had male-biased sex ratios ranging from 10 to 45% females (Hu et al. 1986a), 28–47% (Hu et al. 1986b), and 27–40% (Fuester et al. 1987). In addition, we recently conducted a series of experiments that indicated that lack of a postmating rest period or excessive matings (>2) by females adversely affected the sex ratio of *G. flavicoxis* (Fuester et al. 2003). Even the sex ratios with the highest percentages of females (40–47%) were not as high as those observed in emergence of adults reared from field-collected hosts in India, so there is room for improvement. In this article, we report on the effects of parental age at mating on the reproductive response of *G. flavicoxis*.

### Materials and Methods

**Rearing Procedures.** Cocoons of *G. flavicoxis* from the laboratory culture were isolated in gelcaps so that virgin females were available for controlled matings. Rearings of progeny from virgin females were made to ensure that haploid males are available for controlled matings. Emergees were stored in ventilated cages at 17°C (62°F) until matings were scheduled. Mating was done by placing a virgin female and two to three males in a shell vial. Females were removed after a single copulation and given a 22–26-h rest period before host exposure. Then, each female was placed in a sting unit with five unparasitized fifth instars of *L. dispar* for 24 h, and moved to a fresh sting unit daily until death. Rearings were done at 25°C (77°F), 50–60% RH, and

a photoperiod of 14:10 (L:D) h. Crosses between haploid males and virgin females 0, 1, 4, 9, and 16 d old were made with at least 10 trials for each of the 25 combinations. Postmating longevity, numbers of progeny, and sex ratios (expressed as the percentage of females) of progeny for each female were tracked individually.

**Analysis of Data.** Numbers and sex ratios of progeny produced by females daily and over their lifetimes were recorded, and they were subjected to a two-way analysis of variance (ANOVA) by using the ages of mothers and fathers at the time of mating as grouping factors. The Holm-Sidak multiple comparison procedure (Glantz 2002) was used to isolate groups which differed from one another. Regression analysis was used to see whether progeny production was related to female longevity. We used log-linear analyses to test for differences in proportions of ovipositing females producing mixed and all male progeny.

### Results

**Overall Reproduction, Sex Ratio, and Longevity.** Total progeny produced by females over their lifetimes ranged from 0 to 832 and averaged 265.4. Most of the progeny ( $\mu = 67.4\%$ ) came from hosts offered to females on day 1. Both progeny and sex ratios (proportion females) among progeny produced daily by ovipositing females of *G. flavicoxis* decreased markedly over time (Fig. 1). Only a few females stung for over 10 d, so the elevated sex ratios for days 12 and 13 are based on only a few individuals. Therefore, too much importance should not be attached to reproduction by old females, especially in view of the fact that numbers of progeny produced per day by older females were generally low (<20/d). The number of progeny produced over a female's lifetime was positively correlated with her postmating longevity ( $r = 0.45$ ;  $P < 0.001$ ). Postmating longevity (sum of 1-d holding period and days exposed to host) ranged from 2 to 18 and averaged 6.0 d. Total longevity (sum of pre-mating period, 1-d holding period and days ex-

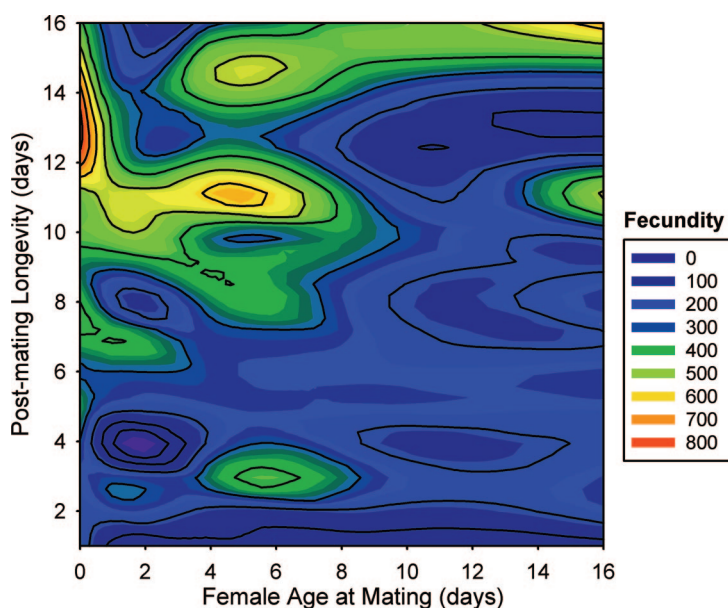


Fig. 2. Influence of premating period and postmating longevity on numbers of progeny produced by females of *G. flavicoxis* over their lifetimes.

posed to hosts) ranged from 0 to 32 and averaged 13.0 d. Sex ratios among progeny produced over the lifetimes of individual females ( $n = 269$ ) ranged from 0 to 74% and averaged 31.6%

**Effects of Parental age at Mating on Female Longevity.** Mean female postmating longevity differed among females mated at different ages ( $F = 3.019$ ;  $df = 4, 264$ ;  $P = 0.018$ ), but the differences were not marked (Table 2), and the only significant difference was between those for females mated at day 4 and day 16.

**Effects of Parental Age at Mating on Progeny Production.** Mean numbers of total progeny produced differed significantly among the different levels of female age at the time of mating ( $F = 7.066$ ;  $df = 4, 242$ ;  $P < 0.001$ ). Females mated four or fewer days after emergence tended to produce more progeny than those mated thereafter (Table 2). Means of total progeny produced by females mated at days 9 and 16 postemergence were significantly lower than those for females mated at days 0 (newly emerged) and 4, whereas the mean number of total progeny produced by females mated the day after emergence (day 1) was intermediate and did not differ from either grouping. Consequently, long premating periods offset the positive correlation between total progeny and postmating longevity to a certain extent. This relationship is illustrated by a contour plot (Fig. 2), which shows that females with short premating periods ( $<9$  d) and long postmating longevity ( $\geq 8$  d) generally had the highest fecundity, whereas those with long premating periods ( $\geq 9$  d) and short postmating longevity ( $<8$  d) had the lowest. Females in other categories tended to have intermediate fecundity.

Male age at mating had no influence on total progeny production ( $F = 0.278$ ;  $df = 4, 242$ ;  $P = 0.892$ ), nor

was there a significant female-male age interaction ( $F = 1.376$ ;  $df = 16, 242$ ;  $P = 0.154$ ). In total, 24 females failed to produce any progeny, but their incidence was not associated with any level of parental age at the time of mating.

**Effects of Parental Age at Mating on Sex Ratios.** Mean sex ratios among progenies produced over a female's lifetime differed among those mated to males of different ages ( $F = 5.218$ ;  $df = 4, 218$ ;  $P < 0.001$ ). Female age at mating had no influence on sex ratio ( $F = 1.898$ ;  $df = 4, 218$ ;  $P = 0.112$ ), and there was no significant female-male age interaction ( $F = 1.469$ ;  $df = 16, 218$ ;  $P = 0.113$ ). Therefore, the data were pooled across female ages at mating. The mean sex ratio was highest among females mated to 1-d-old males, which differed significantly from those mated to newly emerged (day 0) and 9-d-old males (Table 3).

Most of the progeny were produced on the first day females were exposed to hosts (Fig. 1), so we made similar analyses on sex ratios of progeny produced on the first day that females were provided with hosts. The ANOVA results were similar to those of the previous analysis: mean sex ratios for progeny produced by females on the first day of emergence differed among those mated to males of different ages ( $F = 4.472$ ;  $df = 4, 212$ ;  $P = 0.002$ ). Again, female age at mating had no influence on sex ratio ( $F = 2.016$ ;  $df = 4, 212$ ;  $P = 0.093$ ), and there was no significant female-male age interaction ( $F = 1.469$ ;  $df = 16, 212$ ;  $P = 0.183$ ). Overall means were a few percentage points higher, but the ordering of means was virtually the same, being highest among females mated to 1-d-old males, which differed significantly from

**Table 1.** Analysis of variance for mean sex ratios of progeny produced by 0-, 1-, 4-, 9-, and 16-d-old female *G. flavicoxis* mated to 0-, 1-, 4-, 9-, and 16-d-old males on their first day of oviposition

Source of variation	df	Sum of squares	Mean squares	F	P
Male age	4	0.481	0.120	3.036	0.019
Female age	4	0.473	0.118	2.982	0.021
Male age $\times$ female age	16	1.175	0.067	1.694	0.052
Residual	174	6.899	0.040		
Total	198	8.923			

Females with all male progeny excluded.

those mated to newly emerged (day 0) and 9-d-old males (Table 3).

Log-linear analysis indicated that the male age  $\times$  type of progeny (mixed versus all male) interaction was not statistically significant ( $G^2 = 9.356$ ,  $df = 4$ ,  $P = 0.055$ ), but 30% of females mated to newly emerged (day 0) males produced all male progeny; only 10–15% of those mated to older males produced all male progeny.

When crosses (cases) yielding only male progeny were excluded from the preceding ANOVA, both main effects (male age at mating and female age at mating) were statistically significant, but the male age  $\times$  female interaction was not (Table 1). Progeny of females mated to 1-d-old males tended to have higher sex ratios than those mated to males in other age classes (Fig. 3). In addition, females mated the day that they emerged (day 0) tended to have the highest sex ratios (Fig. 3). The sex ratio for the combination of 1-d-old males mated with newly emerged females averaged 60.2% ( $\pm 6.6\%$  [SEM]).

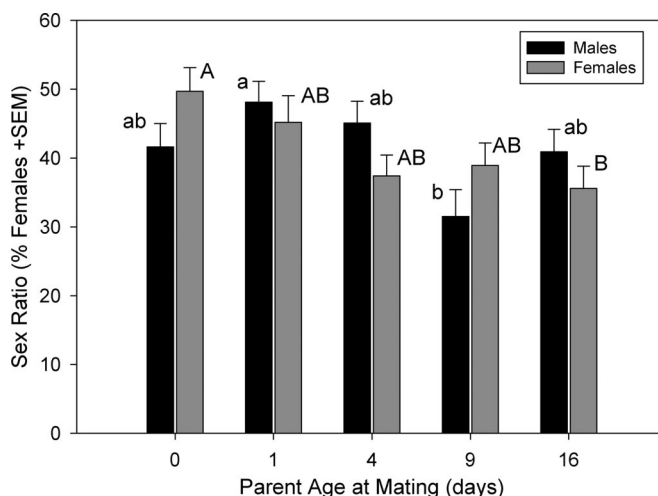
## Discussion

**Longevity of Ovipositing Females.** It was anticipated that means for postmating longevity would be

highest for females mated the day that they emerged and lowest for females mated 16 d after they had emerged, with the values for females mating on intermediate days falling in between. However, there was only one statistically significant difference in mean postmating longevity among ovipositing females mated at different ages, that between the highest (day 4) and lowest (day 16) (Fig. 3), but it was not substantial, the difference being only 2.2 d. Although females stored at 17°C for 16 d before mating had the lowest survivorship, it did not differ significantly from those mated the day that they had emerged, despite the substantial total age difference. Thus, it is clear that the storage procedure successfully diminished the aging process, extending the mean total life span of females stored at 9 or 16 d up to 16 and 21 d, respectively.

**Production of Progeny.** The oviposition pattern exhibited by females of *G. flavicoxis* over their lifetimes (Fig. 1) typified that of species having gregarious development and high fecundity. If presented with sufficient hosts of optimum size, they tend to lay as many eggs as they can as soon as they can. The field dispersal pattern of this species is consistent with this strategy: Krause et al. (1991) found that dispersal of this species was much lower in a high density population of *L. dispar* than in a low density population, suggesting that foraging females were taking advantage of the high density host population by rapidly ovipositing in hosts that were proximal and easy to find, but needed to forage further afield in the low density host population where hosts were sparsely distributed.

Unlike the pattern of postmating longevity, the postmating production of progeny by females over their lifetimes was more in line with what was anticipated. Females mated the day they emerged produced significantly more progeny than those stored



**Fig. 3.** Relationship between sex ratio in progeny produced by a female of *G. flavicoxis* on its first day of oviposition and parental age at mating. Females yielding only male progeny were excluded. Means represented by bars with the same upper case (female parent) or lower case (male parent) letter do not differ significantly ( $\alpha = 0.05$ ). Error bars denote standard error of the estimate.



Table 2. Effects of female age at mating on postmating longevity and total progeny produced of *G. flavicoxis*

Female age at mating (d)	n	Postmating longevity (mean ± SEM)	Total progeny (mean ± SEM)
0	51	5.65 ± 0.55ab	339.1 ± 22.5a
1	42	6.31 ± 0.54ab	291.3 ± 23.8ab
4	61	7.30 ± 0.45a	296.4 ± 19.6a
9	60	5.80 ± 0.49ab	208.5 ± 21.2b
16	55	5.11 ± 0.45b	213.6 ± 20.1b

Means in columns followed by different letters are significantly different ( $P < 0.05$ ) according to Holm-Sidak test.

for 9 or 16 d at 17°C (Table 2). Such substantial differences (>100) suggest that ovisorption, the resorption and use of nutrients in unlaid eggs by females deprived of hosts, could have played a role in diminishing the difference in postmating longevity in females of otherwise disparate ages.

**Sex Ratio.** Because only a fertilized egg give rises to a female individual, the observed reduction in the proportion of females among progeny produced daily as ovipositing females aged (Fig. 1) was consistent with sperm depletion. In this study, females were only allowed to mate once, so it is possible that a different pattern might be observed in field or laboratory situations where males are present, and females could mate later, although a previous study (Fuester et al. 2003) suggested that more than two matings could depress the sex ratio.

Parental age at mating had a highly significant effect on sex ratio, both for progeny produced over a female's lifetime and on the first day of oviposition. The effects of male age at mating were most pronounced (Table 3), with both lifetime and day 1 sex ratios being highest when females were mated to 1-d-old males and lowest when they were mated to newly emerged and 9-d-old males. This was somewhat surprising, because in the laboratory, there is not a pronounced tendency for males of *G. flavicoxis* to emerge a day or so earlier than females (protandry). Therefore, it is the rule for newly emerged females to mate with males that emerged the same day that they do. Yet, the mean sex ratios obtained for matings between newly emerged (day 0) males and newly emerged (day 0) females were only  $21.1 \pm 5.6\%$  (mean ± SEM) and  $26.3 \pm 6.3\%$

Table 3. Relationship between sex ratio in progeny produced by females of *G. flavicoxis* and male age at time of mating

Father's age at mating (days)	Sex ratio (% females) among			
	n	Total progeny over mother's lifetime (mean ± SEM)	n	Progeny (mean ± SEM) on day 1
0	67	25.0 ± 2.8b	63	27.3 ± 3.2b
1	55	41.1 ± 3.0a	47	44.3 ± 3.5a
4	55	34.9 ± 3.0ab	50	39.4 ± 3.5ab
9	37	23.9 ± 3.9b	34	27.4 ± 4.4b
16	53	31.9 ± 3.2ab	48	36.5 ± 3.7b

Means in columns followed by different letters are significantly different ( $P < 0.05$ ) according to Holm-Sidak test.

among progeny produced over the female's lifetimes and on day 1, respectively. It is not known why a substantial proportion of *G. flavicoxis* females mated to newly emerged males failed to produce female progeny, but it might be because spermatogenesis is incomplete when males are very young. Diploid males are known to occur in a number of parasitic Hymenoptera (Stouthamer et al. 1992), although we have not yet demonstrated their presence or absence in this braconid. Moreover, it does not necessarily follow that diploid males would be more prevalent among newly emerged males than 1-d-old males.

A slightly different picture emerged when crosses yielding only male progeny were excluded from the analysis (Fig. 3). The mean sex ratio for day 1 progeny of females mated to newly emerged males no longer was significantly lower than those for females mated to 1-d-old males, but there is no way to predict in advance which males are going to successfully fertilize females and those which will not.

**Implications for Biological Control.** Because both the daily production of progeny and the proportion of female progeny diminish markedly as females of *G. flavicoxis* age, it is most efficient to use only the first day's oviposition in rearing this species. Crosses between newly emerged males and females of *G. flavicoxis*, which occur naturally in laboratory rearings, are clearly counterproductive for the mass rearing of this parasitoid, because newly emerged males seem to be deficient in their fertilization capacity. Because newly emerged females (Fig. 3) produced sex ratios as high or higher as those in other age classes, these would seem to be good candidates for a rearing program. These could be mated with males that emerged the previous day, which consistently gave rise to progeny with good sex ratios in the current study (Table 3; Fig. 3). It is encouraging that progeny resulting from matings between 1-d-old males and newly emerged females averaged >50% females, because almost all of our other trials resulted in lower sex ratios, usually <40% females. We feel that 50% females would be the absolute minimum for a workable sex ratio for commercial production. We have found that isolating cocoons of *G. flavicoxis* in gelcaps is a good way to get virgin females of a certain age class, but this can be a time consuming process. An alternative might be to place newly emerged males in emergence cartons every afternoon increasing the likelihood that they would mate with newly emerged females the next day. Future work with *G. flavicoxis* should focus on determining whether or not there is complementary sex determination (Stouthamer et al. 1992). If so, establishment of pedigrees and crossing to obtain heterozygotes at the sex determination locus might be used to boost sex ratios even higher, although the absolute maximum would seem to be ~75% based on our data.

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